ACTION OF ACETYLCHOLINE, CARBAMINOYL–CHOLINE (DORYL) AND ACETYL–B–METHYL–CHOLINE (MECHOLYL) ON THE HEART OF A CLADOCERAN

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This paper deals with the action of acetylcholine, carbaminoyl-choline, and acetyl-B-methyl-choline on the heart of the cladoceran *Simocephalus vetulus*. Our interest in this work is primarily centered on the comparative physiological evaluation of these chemicals with regard to their stability, potency of action, and their pharmacological effects on the heart as compared with the effects on the intestine of this animal and *Daphnia magna* (Obreshkove, 1941; Mooney and Obreshkove, 1948). A correlation is also made of the observations presented in this paper with certain facts pertaining to the role which tese drugs have been said to play in the transmission of nervous impulses.

METHODS

In this study *Simocephalus vetulus* in their second instar were utilized. The method of rearing and selection of animals for the experimentation as well as the method employed in administering the drugs have been described elsewhere (Obreshkove, 1930, 1941). A single individual in each case was transferred to a micro-culture slide for treatment with the specific drug, and for examination of the activity of the heart under the microscope. After the transference of the animal to the slide, there was usually observed a slight excitation of the heart which persisted for about 15 seconds. One minute was therefore allowed to elapse before the actual readings were taken. Since the animal is seen at all times to ingest particles and fluid with which it comes in contact in the depression slide, it is assumed that the drugs employed in this work were administered orally. The rate of the heart beat was recorded on paper with penicil dots in synchronic rhythm with the heart contractions.

Acetylcholine

It was shown in the study of the effects of acetylcholine on the intestine of *Daphnia magna* and *Simocephalus vetulus* reported previously that when this drug becomes effective, it exhibits its action to the fullest extent with an abrupt, powerful, contractile wave of considerable amplitude. The vigorous peristaltic waves become further intensified with lapse of time, persist for several hours, and terminate in intestinal contracture. The effectiveness of the drug on the heart of *Simocephalus vetulus* is best expressed by the time required for the establishment of the maximum rate of contraction rather than by the time required for the production of an initial effect. In the heart, as in the intestine, there was demonstrated a graded action over a wide range of concentrations. Acetylcholine ranging in concentration from

1×10^{-2} to 1×10^{-9} was employed. In Table I are given the results obtained with 2 concentrations of the drug. It may be seen that when *Simocephalus vetulus* young are treated with acetylcholine, an increase in the rate of the heart beat is observed. Although prolonged consecutive readings were taken for each animal after the application of this and the other drugs employed, in a number of tables presented here, only the maximum heart rate was recorded. With acetylcholine 1×10^{-5} the first evidence of increased cardiac activity appears about 2 minutes after the application of the drug and a maximum rate is established in about 10 to 15 minutes. This is followed by a gradual decline in the heart beat to a subnormal rate which persists for some time. Baylor (1942) also reports a depressing effect of acetylcholine on the heart rate of *Daphnia magna* some minutes after the applica-

TABLE I

Effect of acetylcholine 1 × 10^{-5} and acetylcholine 1 × 10^{-2} on the heart rate of Simocephalus vetulus

Normal rate	Acetylcholine 1×10^{-5}		Normal rate	Acetylcholine 1×10^{-2}	
Beat/min.	Min.	Beat/min.	Beat/min.	Min.	Max. beat/min.
244	2	242	274	5	314
	4	260			
	10	296*			
	15	200			
261	2	282	276	5	314
	5	270			
	10	293*			
	15	207			
277	5	286	264	6	303
	10	295			
	15	309*			
	20	278			
276	4	288	268	5	302
	10	299			
	12	303*			
	15	286			
280	5	278	270	5	318
	10	305*			
	15	194			
275	5	310	272	3	300
	10	301			
	15	315*			
	20	224			
284	5	298	274	4	304
	10	314			
	13	316*			

* Max. rate.

tion of the drug but failed to observe the acceleratory action described here. Stronger solutions of acetylcholine appreciably reduce the time required for the production of the maximum cardiac activity in *Simocephalus vetulus*. Acetylcho-

TABLE II

Effect of prostigmine 1 × 10^{-4} when employed alone and the effect of acetylcholine 1 × 10^{-5} when preceded by 2 minutes treatment with prostigmine 1 × 10^{-4} on the heart rate of Simocephalus vetulus

Normal rate	Prostigmine		Normal rate	Acetylcholine 1 × 10^{-5} after 2 min. treatment with prostigmine 1 × 10^{-4}	
Beat/min.	Min.	Max. beat/min.	Beat/min.	Min.	Max. beat/min.
230	20	268	284	2	321
261	15	294	275	2	320
290	15	319	278	2	325
271	20	329	260	2	306
263	15	298	273	2	309
266	15	294	269	2	297
278	20	308	272	2	303
268	20	299	265	2	294

TABLE III

Effect of mecholyl 1 × 10^{-5} on the heart rate of Simocephalus vetulus following atropine 1 × 10^{-5}*

Normal rate	Atropine 1 × 10^{-5}		Mecholyl 1 × 10^{-5}	
Beat/min.	Min.	Beat/min.	Min.	Beat/min.
272	5	271	2	272
			7	271
			10	269
			30	265
261	5	266	1	262
			3	265
			12	266
			30	262
264	5	263	2	260
			5	262
			10	266
			22	256
286	5	282	2	281
			5	282
			10	278
			15	285
276	5	275	2	282
			4	261
			6	280
			8	268

* Mecholyl—trade name for acetyl-B-methyl-choline.

line 1×10^{-2} produces the maximum effect in a period which varies from 3 to 6 minutes as compared with the 10 to 15 minutes required by acetylcholine 1×10^{-5} (Table I). When acetylcholine (1×10^{-5}) is preceded by prostigmine (1×10^{-4}) the maximum effect appeared in about 2 minutes (Table II) in comparison with the 10 to 15 minutes when acetylcholine 1×10^{-5} was employed alone. A similar action was demonstrated for physostigmine in the production of an intensification of the Doryl and Mecholyl effects. The observations are in accord with those made on the intestine of this animal (Mooney and Obreshkove, 1948). Likewise when prostigmine was administered alone, it produced the same effect as acetylcholine but required on the average a longer period of time for the production of the maximum effect (Table II).

TABLE IV

Effect of Doryl on the heart rate of Simocephalus vetulus; abolishing of this effect by atropine; and reestablishment of the Doryl effect*

Normal rate	Doryl 1×10^{-10}		Atropine 1×10^{-5}		Doryl 1×10^{-5}	
Beat/min.	Min.	Maximum beat/min.	Min.	Beat/min.	Min.	Maximum beat/min.
284	3	323	2 3	271 273	3	301
261	2	290	2 3	263 265	3	284
268	4	287	1 5	266 251	3	293
265	3	290	2 3	250 253	3	293
273	1	295	2 4	282 270	3	288
281	3	296	1 2	261 263	3	304
281	3	308	2 5	304 270	3	292
269	1	298	2 5	283 260	1	288
276	3	304	1 4	297 275	2	299
266	3	304	1 4	285 271	2	298

* Doryl—trade name for carbaminoyl-choline.

Acetyl-B-methyl-choline (mecholyl)

Acetyl-B-methyl-choline exerts a characteristic effect on the heart of *Simocephalus vetulus* in eliciting a cardiac acceleration in a manner similar to that of acetylcholine. It appears that this drug is more potent on the heart than acetylcholine as judged by the time required for the production of the maximum excitatory action when drugs of the same concentration are employed. A very clear antagonism was found to exist between acetyl-B-methyl-choline and atropine. In a series of 5 experiments a preliminary application of atropine 1×10^{-5} for 5 minutes prevented the appearance of the characteristic effect ascribed to Mecholyl even 30 minutes after the application of the latter drug (Table III). The same concentration of atropine was found effective when added after Mecholyl. These observations are similar to those reported for the intestine of this animal.

TABLE V

Effect of Mecholyl 1×10^{-5} on the heart rate of Simocephalus vetulus and the abolishing of this effect by atropine 1×10^{-5}*

Normal rate	Mecholyl 1×10^{-5}		Atropine 1×10^{-5}	
Beat/min.	Min.	Max. beat/min.	Min.	Beat/min.
260	3	293	2 3	264 258
277	3	302	1 3	275 278
289	3	308	1 3	284 286
282	4	306	1 2	283 280
271	1	305	1 3	284 280
268	1	301	2 3	267 270
270	2	316	2	278

* Mecholyl—trade name for acetyl-B-methyl-choline.

Carbaminoyl-choline (doryl)

The heart of *Simocephalus vetulus* responds even to a very weak solution of carbaminoyl-choline. A maximum rate of heart beat is produced by a low concentration of this drug (1×10^{-10}) in a comparatively short period of time. Whereas atropine was shown to abolish rapidly or prevent completely the action of acetyl-B-methyl-choline, when the doryl effects are abolished by atropine, they are quickly reestablished after the atropine treatment is followed by carbaminoyl-choline (Table IV). Prolonged washing reduces and in time completely abolishes the effects of carbaminoyl-choline and of all the other drugs employed in this work. In abolishing the action of the drugs, the effects of washing were found to be considerably slower than those produced by atropine.

TABLE VI

Action of Doryl 1 × 10^{-5} followed by washing with distilled water on the heart rate of Simocephalus vetulus (second day young)

No.	Normal	Doryl 1 × 10^{-5}%		Distilled H_2O	
	Beats/minute	Minutes	Beats/minute	Minutes	Beats/minute
1	238	2½ 5	289 285	2 5 10 15 20	281 279 258 257 250
2	256	2½ 3½	294 295	2 4 6 10 15	290 291 289 276 270
3	282	2 2	301 307	2 4 10 15	304 300 287 288
4	277	2 3	298 301	2 4 10 15	299 301 285 272
5	269	2 3½	296 303	2 4 10 15	294 291 274 270

DISCUSSION

Our endeavor to demonstrate the nerves distributed to the heart of *Simocephalus vetulus,* so important for the further clarification and analysis of the results presented here, was beset with many difficulties. It was demonstrated earlier, however, that if the intestine of *Daphnia magna* is touched with a fine glass needle at the bend of the digestive tube where the stomach enters the intestine, the heart immediately ceases to beat and after a certain period, depending on the degree of the mechanical stimulation applied, the heart escapes from inhibition (Obreshkove, 1942). The heart in this respect behaves similarly to the inhibition produced after electrical excitation of the vagus nerve in vertebrates. When *Daphnia magna* was treated with acetylcholine, prior to the production of inhibition or during the heart inactivity, the period of cardiac inhibition was observed to be considerably shorter. It must be pointed out that whereas acetylcholine has been shown to produce cardiac excitation in some invertebrates, in others it produces inhibition. Prosser (1942) after an extensive review of the literature presents evidence to indicate that the invertebrate hearts may be grouped into three classes with respect to the action of acetylcholine and suggests that those hearts which are accelerated by the drugs are neurogenic, those which are inhibited are myogenic and those which are unaffected by acetylcholine are non-innervated.

Acetylcholine, acetyl-B-methyl-choline, and carbaminoyl-choline produce cardiac excitation in *Simocephalus vetulus*. This action of the drugs is antagonized by atropine and augmented by prostigmine and physostigmine. These and the other observations recorded in this paper are of such a nature as to suggest strongly some known facts pertaining to the role which acetylcholine and other cholinergic drugs have been said to play in the transmission of nervous impulses. The demonstration by Artemov and Mitropolitanskaja of an acetylcholine-like substance in Daphnia (1938) adds further interest to the problem.

SUMMARY

1. Acetylcholine, carbaminoyl-choline and acetyl-B-methyl-choline produce in *Simocephalus vetulus* cardiac excitation. The heart acceleration in each case was followed by inhibition.

2. This action of the drugs is antagonized by atropine and augmented by prostigmine and physostigmine.

3. Acetylcholine exhibits a graded action for a wide range of concentrations (1×10^{-2} to 1×10^{-9}) as revealed by the time required for the production of a maximum cardiac excitation.

4. In contrast with carbaminoyl-choline, a marked antagonism was found to exist between acetyl-B-methyl-choline and atropine. When atropine is followed by carbaminoyl-choline, the Doryl effect appears in comparatively short periods of time.

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